Dengue: a trilogy of people, mosquitoes and the virus. Current epidemiology and pathogenesis in (non-)endemic settings
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Dengue is the most common vector-borne viral disease worldwide, and is ranked among the most important infectious diseases by the World Health Organization (WHO). Also in Vietnam, dengue poses a major challenge to public health. The studies presented in this thesis addressed the epidemiology and disease transmission of dengue (chapter 2-8), and the clinical and vial pathology (chapter 9-11).

THE PROBLEM OF DENGUE

Humans are the primary host of dengue virus (DENV) and transmission of DENV takes place through the bite of the principal mosquito vector, *Aedes aegypti*. Dengue is difficult to grasp as a clinical entity. It usually presents as a highly unspecific illness and is hardly recognized as a clinical entity by primary health care physicians. The large burden of dengue (chapter 2, 3, 4), the relatively low complication rate (chapter 6 and 9) and the difficulties in predicting severe disease make it almost impossible for the primary health services in developing countries to tailor the care to those who need it, while giving care to all is unaffordable. Inabilities to make an early diagnosis and to identify those who are at highest risk to progress to severe complications in the early phase have impeded novel treatment interventions. Advancement in understanding of dengue pathogenesis (e.g. antibody dependent enhancement, differences of serotypes or genotypes, viral load, antigenemia and cytokine profile) and perhaps the identification of new prognostic markers (biomarkers, host genetics) may improve case management and treatment intervention strategies. For the near future, health care providers will have to rely on predictive algorithms which include a combination of clinical symptoms, signs, and laboratory parameters such as haematological profile, viremia, NS1 antigenemia to identify severe dengue.

Disease control is mainly based on tackling the vector and elimination of environmental risk factors. Vector control strategies include environmental changes (improved water supply, emptying or covering tanks, and underground reservoirs), personal protection (protective clothing, mats, and nets), biological control (fish, bacteria) and chemical control (insecticide). However, these tools have not been implemented vigorously and are unsatisfactory for reduction of transmission. More systematic insight is needed in the strengths and weaknesses, opportunities and threats of the implementation of the dengue control program at provincial, district and community level is needed. Although some environmental risk factors (i.e. use of a pit latrine and littering with discarded cans and having pigs on domestic setting) were identified to be associated with dengue antibody prevalence in children in Binh Thuan province and described in chapter 3, disease control by means of sanitary measures may be extremely difficult, and it may even increase the risk of major epidemics by decreasing basic reproduction number ($R_0$).

DENGUE EPIDEMIOLOGY, BURDEN OF DISEASE AND TRANSMISSION

Dengue has a wide spectrum of clinical presentations, often with unpredictable clinical evolution and outcome. Dengue fever can easily be confused with other infectious diseases, especially in the early phase of disease. Communicable diseases, including dengue constitute a substantial part of the health problems in Vietnam. Dengue among AUF was a very common disease, with major impact on public health in Binh Thuan province, affecting mainly children and young adolescents (chapter 2 and 9). The majority of patients with (uncomplicated) dengue were not recognised as such, which lead to substantial under-reporting of dengue in the health information system, as reporting is usually based on clinical signs and symptoms. Patients with severe symptomatic dengue are routinely reported to the Provincial Center for Preventive Medicine, mostly without laboratory confirmation. Because routine notification data grossly underestimated the true incidence of dengue, chapter 3 and 4 described two methods to estimate the seroconversion rate, which corresponds to the true incidence rate of first infections. The incidence of first infections is a good indicator of the real infection pressure in the community. Similar to other dengue endemic areas of Southeast Asia, dengue is mainly a childhood disease and children experience an annual exposure risk (incidence or $R_0$) of ~10%. The relatively high estimates of $R_0$ estimates and the gradual increase in sero-prevalence with age, suggest that dengue in in Binh Thuan province is already hyper-endemic.

LOCALLY ACQUIRED DENGUE

Despite the wealth of knowledge on many aspects of the biology of dengue, patterns of dengue transmission at the village (chapter 5) and provincial level (chapter 7) remains scarce. It is known that children and young adolescent are most affected (also chapter 2, 3, 4, 6 and 9), but it is uncertain if these infections occur at home, at school, or elsewhere. In chapter 5, we attempt to address this issue by demonstrating that new DENV infections occurred near places where sero-prevalence was highest at baseline. This point at important spatial heterogeneity in the transmission of dengue. Undiagnosed asymptomatic DENV infections or unrecognized dengue patients with mild symptoms may be more important for the spread of DENV than symptomatic and notified cases. Undetected persistence of local transmission may have implications for future deployment of prevention strategies.

Furthermore, movement of dengue infected humans is thought to play one of the most important role in dengue transmission, since the *Aedes* vector has a restricted flight range (< 150 m). Movement and transport of humans or vectors at a national, regional or international scale creates new opportunities for vectors to establish in permissive environments, and for virus to be transmitted where competent vectors exist. Inadequate or interrupted vector control activities, determined by economic and political priorities will directly affect individuals’ risk of exposure to infection in endemic settings. These social,
demographic and economic drivers that are thought to be responsible for much of dengue’s expansion and intensified transmission over recent decades, are not easily to overcome. Among all the barriers that obstruct reduction of transmission, an underestimated issue is the socio-cultural context of dengue. Risk perceptions among people remain unclear and people are reluctant to change behaviour. Since dengue transmission is such a multifactorial problem, multifaceted research approaches are probably the most effective. Participatory research could gain insight in the missing link between human risk perceptions, attitude and behaviour and the poor results of dengue transmission reduction.

TRANSMISSION DYNAMICS

Dengue transmission in endemic settings is characterized by nonlinear dynamics over time, with strong seasonality, multi-annual oscillations and irregular temporal fluctuations in incidence. Besides the seasonality of dengue transmission, periodic epidemics and more irregular intervals of outbreaks are commonly observed. Multiple factors may influence the dynamics of dengue including environmental and climate factors, host-vector interactions and the population-wide immune landscape. Climate variability is postulated to be an important determinant of dengue epidemics. Wavelet analysis has been demonstrated to be suitable for investigating time series data from non-stationary systems and for inferring associations within such systems. In chapter 7, we provide insights into the persistence and spatial spread of dengue throughout Binh Thuan province, southern Vietnam. We found that the multi-annual wave of dengue infection was moving towards Phan Thiet district and might originate from another, but nearby epicenter. Whether the multi-annual periodicity of dengue incidence exists or not, the detection of periodic cycles for dengue transmission is characterized by nonlinear dynamics over time, and irregular intervals of outbreaks are commonly observed. Multiple factors may influence the dynamics of dengue including environmental and climate factors, host-vector interactions and the population-wide immune landscape (reviewed in chapter 8). Climate variability is postulated to be an important determinant of dengue epidemics. Wavelet analysis has been demonstrated to be suitable for investigating time series data from non-stationary systems and for inferring associations within such systems. In chapter 7, we provide insights into the persistence and spatial spread of dengue throughout Binh Thuan province, southern Vietnam. We found that the multi-annual wave of dengue infection was moving towards Phan Thiet district and might originate from another, but nearby epicenter. Whether the multi-annual periodicity of dengue incidence exists or not, the detection of periodic cycles for dengue incidence itself provides no insights into the processes that cause dengue incidence oscillations (e.g. environmental and climate factors, host-vector interactions and population herd immunity). Further extensive collection of and analyses on surveillance data from other provinces in southern Vietnam are pending to test this hypothesis.

HOST DETERMINANT OF CLINICAL OUTCOMES

Two key risk factors for both symptomatic and severe dengue disease are 1) the age at infection and 2) the acquisition of secondary infections. A recent study in Brazil investigating the relationship between age at primary infection and the risk of febrile illness, suggested that adults are more likely than children to have clinical dengue. Although age at infection and infection parity are the representative key modulators of clinical dengue and disease severity, their relationship was established and explicitly quantified in chapter 6, by analysing epidemiological data sets in southern Vietnam. For both primary and secondary infections, higher age at DENV infection was shown to result in higher risk of clinical attack. Age as an important modulator of clinical dengue explains the recent increases in dengue notifications rates in ageing countries in Southeast Asia, and moreover, poses a paradoxical problem of an increase in the incidence among adult patients resulting from a decline in the force of infection. It should be noted that only a small proportion of patients with secondary DENV infection (and an even smaller proportion with primary infection) develop severe dengue. Our current knowledge on the relationship between age and symptomatic dengue merits additional studies on the clarification on the population impact of age-specific risks of clinical attack on the total number of severe forms of dengue.

PLASMACYTOSIS IN DENGUE

One of the characteristic features of dengue is the occurrence of leucopenia and thrombocytopenia, probably resulting from virus induced bone marrow suppression. Despite the general bone marrow suppression, blood plasmacytosis has been reported in a few patients with DENV infection. Blood plasmacytosis is an unusual haematological finding that is most commonly seen in plasma cell leukaemia or advanced stage multiple myeloma, in which case the plasma cells are part of the malignant clone and thus are monoclonal. Non-malignant reactive peripheral plasmacytosis is occasionally found in a variety of diseases. Polyclonal peripheral blood plasmacytosis has occasionally been described in dengue virus (DENV) infected patients. In chapter 10, we showed that blood plasmacytosis is a common event in DENV infection, which is characterized by a transient presence of polyclonal PCs in the circulation. In this specific context, an associated plasmacytosis will be seen more frequently in travelers with dengue and in dengue endemic areas. In resource poor settings, peripheral blood smears (performed for malaria diagnosis or other conditions) may demonstrate extreme peripheral plasmacytosis. Peripheral plasmacytosis will resolve quickly (< 14 days), if dengue is the underlying illness. It should be emphasized that recognizing this self-limited phenomenon could obviates extensive clinical evaluation.

Indeed, the mechanism, contribution and possible role of peripheral plasmacytosis in dengue pathogenesis remain unclear and deserve future investigation. Important questions are whether active DENV replication takes place in peripheral PCs and whether PCs are redistributed and released from the bone marrow into the circulation or whether they represent an increased production and if this is virus or cytokine driven?

VIRAL DIVERSITY IN DISEASE SEVERITY

The basis for the genetic diversity in DENV is its error-prone RNA polymerase, such that mutations commonly occur during viral replication. DENV therefore exists as a population of closely related sequences and this degree of intra-host genetic diversity has been proposed to have implications for pathogenesis of DENV infection, disease outcome, virus evolution, and host immunity.
In contrast to some previous studies we observed no relationship between the extent and pattern of DENV-1 genetic diversity and disease severity, immune status, or level of viremia (chapter 11). We also showed that nucleotide sequence diversities of viral populations were very low. Despite such sequence conservation, we observed clear evidence for mixed infection, with the presence of multiple phylogenetically distinct lineages present within the same host and we likely greatly underestimate its true frequency as we cannot identify mixed infection among very closely related viral lineages. Mixed infection is a potentially important contributor to intra-host virus genetic and phenotypic diversity, and provides the raw material for intra-serotype recombination. However, we were unable to determine whether these mixed infections represent the simultaneous infection (i.e. co-infection) or superinfection of multiple viral lineages in humans. This is clearly an area that requires additional study.

FUTURE PERSPECTIVE

Current research priorities for dengue are to improve case-management and prevent progress to severe disease and mortality, to enhance understanding of dengue pathogenesis and to reduce dengue virus transmission and to conduct policy research that contributes to an adequate public health response. This thesis contribute to advancing the understanding of different aspects of the burden of disease, its epidemiology and disease dissemination/transmission and described clinical observational studies for a better understanding of dengue pathogenesis. However, many questions remain unanswered and merit further investigation. Table 1 summarizes questions derived from this thesis for future research.

<table>
<thead>
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<th>Table 1. Research questions derived from this thesis.</th>
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<tbody>
<tr>
<td><strong>Epidemiology and burden of disease</strong></td>
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<tr>
<td>How will high risk areas at community level be identified? Will identification of high risk areas (through sero-surveillance in children and GIS) plus vector control measures reduce the burden of disease and dengue spread/transmission?</td>
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<tr>
<td>How and to what extent do asymptomatic infected individuals and patients with mild dengue contribute to transmission patterns? How can asymptomatic infected individuals or patients with mild dengue be identified?</td>
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<td>What is the age-specific proportion of severe dengue patients among the proportion with symptomatic dengue?</td>
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<td>What are the dengue transmission dynamics in southern Vietnam at district and provincial level?</td>
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<td>Does the multi-annual transmission cycle eminates from Ho Chi Minh City? What is the influence of Thailand and Cambodia on dengue transmission in southern Vietnam? What is the influence of local climate on transmission dynamics?</td>
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<tr>
<td><strong>Pathogenesis</strong></td>
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<tr>
<td>What is the contribution of virus virulence (serotype, genotype or lineages) to disease severity? What is there impact on age at infection?</td>
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<tr>
<td>Does DENV replication takes place in peripheral PCs? Are PCs redistributed and released from the bone marrow into the circulation or increased production? Is this phenomenon virus or cytokine driven?</td>
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<tr>
<td>What is the extent of genetic diversity of DENV in the vector (A. aegypti and A. albopictus)? What are the statistical methods to determine whether a mixed infection of multiple viral lineages represent co-infection or superinfection?</td>
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CONCLUSION

Dengue remains an enormous public health threat globally and in Vietnam which will remain a major public health issue for the coming decades. The disease burden poses major pressure on health care services and has social and economic implications. Development of public health intervention tools are needed and may require community participation for containment of dengue transmission. Knowledge of the virulence of the virus including sequential infection, host factors, host immune response on dengue pathogenesis may promote treatment and intervention strategies.